GUIDE TO THE USE OF THE
MOSQUITO FISH, GAMBUSIA AFFINIS,
FOR
MOSQUITO CONTROL

Navy Environmental and Preventive
Medicine Unit No. 2
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A GUIDE TO THE USE OF THE MOSQUITO FISH, GAMBUSIA AFFINIS, FOR MOSQUITO CONTROL[1,2]

by

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[1]*The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or as reflecting the views of the Navy Department or the naval service at large.*

[2]*Mention of a pesticide or a proprietary product in this paper does not constitute a recommendation or an endorsement of the item by the authors or the U. S. Navy.*

The mosquito fish, Gambusia affinis; Male (above) and Female (below).

PREFACE

The last five years have witnessed a renewed interest in the use of fish as a supplementary measure to chemicals in controlling mosquitoes. Based on past evidence, there is little doubt that the mosquito fish, Gambusia affinis, can be used successfully in suppressing mosquito populations; an added benefit being a reduction in the quantity of pesticides applied to the environment. Mosquito-eating fish are not, however, the ultimate answer in mosquito control, and can never replace chemicals for every situation.

Whether the control measure is chemical or biological, the characteristics and limitations of the agent must be thoroughly understood. A mosquito fish program will fail if the biology and ecological requirements of the fish are not considered. Also, the impact of the fish on the aquatic environment cannot be underestimated as there is good evidence that the indiscriminate use of mosquito fish can be as detrimental as the misuse of pesticides.

The purpose of this guide is twofold: (1) to provide a compact reference on the bionomics and husbandry of mosquito fish; and (2) to provide guidelines in establishing an effective and conscientious mosquito fish program which is based on sound ecological considerations.

Information for this guide was drawn from three sources: (1) professional papers on the subject of larvivorous fish; (2) data from leading authorities; and (3) actual field and laboratory observations. While extensive literature has been published on the biology of Gambusia, it is not always readily available to those in the field. Therefore, the section entitled Biology is purposely detailed allowing the investigator to become more familiar with this unique fish. The techniques and procedures in the Husbandry section can be adapted to large or small operations; selection of the most suitable methods is left to the judgement of the individual. The bibliography, while not exhaustive, will serve as a supplementary guide for the investigation of particular points of interest.

This guide will be periodically revised and updated as additional information becomes available.

ACKNOWLEDGMENTS

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INTRODUCTION

The use of fish for mosquito control is not a new concept. The technique was first seriously investigated in the early 1900's after it was discovered that malaria was transmitted by mosquitoes. A continuing interest in larvivorous fish suffered with the advent of DDT and subsequent fast-acting residual insecticides. Recently, however, renewed attention is being given to fish as biological control agents. Two reasons for this change in attitude are the greater public concern over unintentional effects of pesticide contamination on the environment and the fact that chemical control with insecticides has become more costly, shorter-lasting and, owing to the evolution of resistant mosquito strains, less effective (Bay, 1967a).

In an annotated bibliography, Gerberich and Laird (1968) list 686 papers dealing with the use of fish for mosquito control. While over 200 different fish species are mentioned, 279, or 41%, of the references cited deal partly or entirely with only one species: the mosquito fish, Gambusia affinis. The southeast Asian Panchax group and the common guppy, Lebistes reticulatus, are the closest runners-up, accounting for 8.6% and 6.5% of the literature, respectively. Bay (1967b) states that Gambusia affinis has become almost synonymous with biological control of mosquitoes and is, in fact, one of the most widely disseminated of any biocontrol agent. From an immediate and practical standpoint, mosquito fish offer great potential as an adjunct to chemicals for mosquito control. It is on this species, therefore, that we focus our attention.

The development of a mosquito fish program should, from the onset, be a cooperative effort between medical department and public works personnel and, when available, with individuals assigned fish and wildlife duties for the activity. For maximum impact, the program might also be coordinated with local state and county health departments, particularly those involved in mosquito control. Assistance with the program can be obtained from Navy entomologists at a Navy Environmental and Preventive Medicine Unit, a Navy Disease Vector Ecology and Control Center or a Field Division of the Naval Facilities Engineering Command. Since most states have regulations concerning the importation of certain fish species, it is mandatory that the state Fish and Game Commission or their military representative be contacted when interstate shipment of

Gambusia is contemplated. Additional advice may also be obtained from the U. S. Fish and Wildlife Service and most state universities.

HISTORY

Baird and Girard first described Gambusia affinis in 1854. However, it was not until the early 1900's that their usefulness as mosquito predators become well established. During that period, the first long distance transplantation of mosquito fish was made. At the request of the Hawaii Agriculture Experiment Station, about 150 Gambusia were brought from Seabrook, Texas to Honolulu in 1905 by Mr. Alvin Seale. The fish flourished and by 1907 they had already established a record of being extremely effective mosquito predators (Rockefeller Foundation, 1924; Krumholz, 1948). In 1913, Seale (1917) transported 24 Gambusia in a glass jar in his stateroom from Hawaii to the Philippine Islands where they also thrived and reproduced in large numbers. From the Philippines, they were eventually introduced into areas of Indochina, Japan and China. In 1921, at the request of the International Red Cross, stocks of Gambusia were sent from Augusta, Georgia to Spain by the U. S. Bureau of Fisheries (Rockefeller Foundation, 1924; Krumholz, 1948). Gerberich and Laird (1968) consider this event to be significant as it eventually became one of the earliest and most widespread uses of a biological control agent in the medical entomology field. Italy imported Gambusia from Spain in 1922 and from this Italian stock, distribution was soon extended to countries throughout Europe including Yugoslavia and the USSR (Transcaucasia Region) in 1924, Algeria and Corsica in 1926, and Greece in 1928. From Corsica, mosquito fish were imported to Egypt and then to Cyprus, Syria and the Sudan. Finally, through the support of the Rockefeller Foundation, Gambusia were established in Mexico, Central and South America to combat container-breeding mosquitoes in a drive against yellow fever (Gerberich and Laird, 1968). During yellow fever control efforts in Key West, Florida, Gambusia were used successfully in place of oil for the control of Aedes aegypti in cisterns (LeVan, 1941).

Gambusia affinis also received widespread distribution and use in malaria control. In the United States, Hildebrand (1921) reported that the use of mosquito fish during 1920 malaria control campaigns was responsible for producing more permanent results in many southern states at a

reduced cost. It is also of interest that during this time the Health Department in Richmond, Virginia, stocked all of its fountains, reservoirs and lakes with Gambusia and established hatcheries to supply mosquito fish to any community in the state that requested them (Rockefeller Foundation, 1921 and 1924). In other regions, the New Guinea Medical Service provided local distribution of Gambusia in 1930 which later were reported to be the most successful antimalarial measure taken in the Territory (Ford, 1949). The Canary Islands imported Gambusia from Spain in 1943 to control an outbreak of malaria (Gerberich and Laird, 1968), and during World War II, U. S. Army Malaria Control Units utilized Gambusia in the Pacific to control anopheline breeding in wells and cisterns (Krumholz, 1948; Simmons, 1949). More recently, Tabizzadeh, et al., (1970), reported the extensive use of Gambusia in Iran for malaria control. In California (which originally imported Gambusia from Texas in 1922), successful control of the important disease vectors, Culex tarsalis and Anopheles freeborni, was achieved with the use of mosquito fish (Hoy and Reed, 1970; Hoy et al., 1971).

BIOLOGY

Taxonomy

Gambusia affinis (Baird and Girard) is one of some 150 species of fish in the family Poeciliidae. Members of this family are all from the New World with the majority found in Central America and the West Indies (Rosen, 1972). The etymology of the name Gambusia comes from "Gambusino", a provincial Cuban word which denotes "nothing". Thus, it is said, "to fish Gambusinos is to catch nothing". The species name, affinis, means "neighboring" or "related". Because of its well known appetite for mosquito larvae, the common name usually applied to the species is "mosquito fish". However, it may also be referred to in the literature as a "top minnow". due to its habit of seeking food at the surface.

A relatively flat head, small body and protrusible mouth are characteristic of mosquito fish. At birth, and for some time afterward, the anal fin of both sexes is similar. However, sexual dimorphism is later exhibited as the fin becomes small and rounded in the female while that of the male is modified into an elongated, rod-shaped copulatory organ, the gonopodium (Figure 1). The gonopodium is normally carried backward and

parallel to the body, but it can be quickly moved at any angle, forwards or sideways. Adult females range from 1 to 2 1/2 inches in length while the mature males average about 1 1/4 inches. According to Krumholz (1948), female Gambusia attain greater lengths than males since they continue to grow until death. Males, however, grow very little after the gonopodium is completely formed. In both sexes, the body is pale grey with a blue metallic sheen. The belly is silver and the back, or dorsum, is brown or olive green. In many cases, there is a dark, transverse bar across the eye. Female Gambusia and guppies are similar in appearance, but Gambusia can be distinguished by the rows of tiny black dots on the dorsal and caudal fins (Figure 1); a characteristic found lacking in guppies. Another good field characteristic is that in female Gambusia, the first ray of the dorsal fin is posterior to that of the anal fin, while in the guppy they are almost in direct line with one another.

Two subspecies of Gambusia affinis occur that are essentially identical in color, form, size and habits: Gambusia affinis affinis and Gambusia affinis holbrooki. The two can be separated by the fact that G. a. holbrooki has eight rays in the dorsal fin while G. a. affinis has only seven. A velvet black spotting or blotching may be present in either subspecies. Such individuals are not different species, but are merely showing a melanistic or pigmented expression such as occurs in "marbled mollies", a fish well known to tropical fish hobbyists. This phenomenon is rarely seen in females but may occasionally occur in the males.

Distribution

Because of its use for mosquito control, Gambusia affinis probably has the widest range of any fresh water fish. It is found in nearly all of the warmer regions of the world and has been successfully established in areas where relatively severe cold weather occurs, such as Utah (Rees, 1934 and 1945), Michigan (Krumholz, 1944), Alberta, Canada (Mail, 1954) and the USSR (Krumholz, 1944). In the U. S., Innes (1971) divides the distribution of Gambusia affinis between the two subspecies. The range of the eastern subspecies, G. affinis holbrooki, is given as Delaware to Florida and Alabama, whereas the western subspecies, G. affinis affinis, ranges from Alabama to Illinois and south to the Texas coastal region. In Alabama, the two subspecies reportedly meet and their distinctive features are lost. It is likely that this

phenomenon occurs elsewhere as Gambusia is found in Arizona, California, Colorado, Kansas, Massachusetts, Nevada, New Jersey, New York, New Mexico, Ohio, Washington, Wisconsin, and probably many other states.

Habitat

In areas where it is native or has become well established, Gambusia affinis is found in all sluggish or standing lowland waters which are accessible to it through natural channels. It thrives in a wide variety of water types including fresh or brackish, clear or muddy, shallow or deep. Gambusia is seldom found in swift-flowing streams or water that is polluted with chemical wastes acid in nature, but it can tolerate water moderately polluted with sewage. When larger fish are not present, Gambusia may be found in deep water. However, it shows a distinct preference for shallow water where it is protected from predaceous fish and where food and vegetation are more abundant. Kuntz (1913), in fact, observed large numbers of mosquito fish in water less than 1 inch in depth.

Although essentially a warm water fish, Gambusia can tolerate a wide range of temperatures. Hildebrand (1925) reported mosquito fish withstanding water temperatures of 102° F. in nature. Conversely, relatively cold water strains have been developed and have been reported to successfully overwinter under ice in certain areas of Utah (Bay, 1967a), Michigan and Illinois (Krumholz, 1944), and Manitoba, Canada (Smith, 1960). It should be noted that during winter, the fish hibernate in the lower water depths and are rarely seen until the spring when water temperatures become increasingly warmer.

It is well known that Gambusia can inhabit areas seriously deficient in dissolved oxygen for short periods of time without apparent stress. This ability is attributed by Lewis (1970) to a morphological adaptation of the fish to oxygen depletion. Specifically, their dorsally oriented mouths and flattened heads enable them to effectively utilize the oxygen-rich water at the air-water interface without dramatically altering their usual swimming posture. Gambusia are, therefore, suited for occupying habitats subject to periodic oxygen depletion such as in sewage lagoons.

Feeding Behavior

Gambusia feed on a number of aquatic organisms including mosquitoes. They are, in fact,

voracious feeders that consume a wide variety of phytoplankton, zooplankton, as well as some of the larger aquatic insects (Mulla and Isaak, 1961). Hildebrand (1921) found that while insects constitute a large part of the diet, a majority of the Gambusia studied also consumed some plant tissue consisting mostly of algae. In determining what organisms will be taken as food by Gambusia it would appear that availability is more important than choice. Studies in Alabama by Hess and Tarzwell (1942) demonstrated, for instance, that when mosquito populations increased, the number of Gambusia eating them, as well as the number eaten per fish, also increased. By analysis of stomach contents, it was also found that a peak in feeding activity occurred soon after daylight with a decline to a minimum in the later afternoon and then an increase to a second, but lesser peak, in the evening. Hubbs (1971) found similar peaks to occur at 1100 and 1900 hours in Texas. Gambusia searches for food at or near the water surface, but will search the bottom if food is scarce elsewhere.

Mosquito fish are highly attracted by prey movement. Hildebrand (1921) reports their range of vision to be only 4 to 5 inches, and the movement of larger prey is more quickly noticed than small prey. Anopheles larvae will, in fact, remain motionless when fish are nearby. According to Hildebrand (1919), such a protective instinct is probably highly developed in mosquito larvae since "inactivity" is the only protection they have against their fish predators. The fact that Gambusia show increasing preference for later instars and even greater predilection for pupae has been reported by a number of investigators (Hess and Tarzwell, 1942; Mulla and Isaak, 1961; Harrington and Harrington, 1961).

The voracious appetite Gambusia have for mosquitoes has been reported on several occasions. Hildebrand (1921) observed one large female to eat 225 larvae and pupae within a 1 hour period. In another instance, a pair of half grown Gambusia consumed over 5,000 larvae in 11 weeks (Seale, 1917). All sizes and ages of Gambusia readily feed on mosquito larvae, and even fry only a few hours old will attack young instars. Gambusia are also cannibalistic and will prey on their own young if given the opportunity.

Reproduction

Oviparous or egg-laying fish generally produce thousands or millions of unprotected eggs, but relatively few survive. Gambusia which are ovoviviparous (livebearers), give birth to smaller

numbers, but the newborn are in a more advanced stage of development and better adapted to begin the struggle for existence than most fish hatched from eggs.

After mating, a female **Gambusia** has the ability to store the sperm and deliver a number of successive broods without further contact with a male. Mating is strictly promiscuous and pairs are not formed as in many egg-layers. The fertilized eggs hatch within the body cavity of the female and the young lie folded with head and tail meeting. They are delivered in this form at birth; either one or two at a time. Almost immediately, they straighten out and swim to the nearest refuge available. At birth, they are approximately 3/8 inch in length, and the ratio of males to females is essentially 1:1. As the fish grow older, this ratio gradually favors the females since they are hardier and live longer than the males (Krumholz, 1948).

A gravid or sexually mature female is easily recognized by the distended abdomen and a large, black triangular area in front of the anal fin known as the "gravid spot" (Figure 1). This characteristic is due to black pigment in the peritoneum (a thin membrane which lines the abdominal wall and various organs) which shows through the distended abdominal wall of the pregnant female. The pigmented area is also found in immature females, but it reaches its maximum size just before the female gives birth.
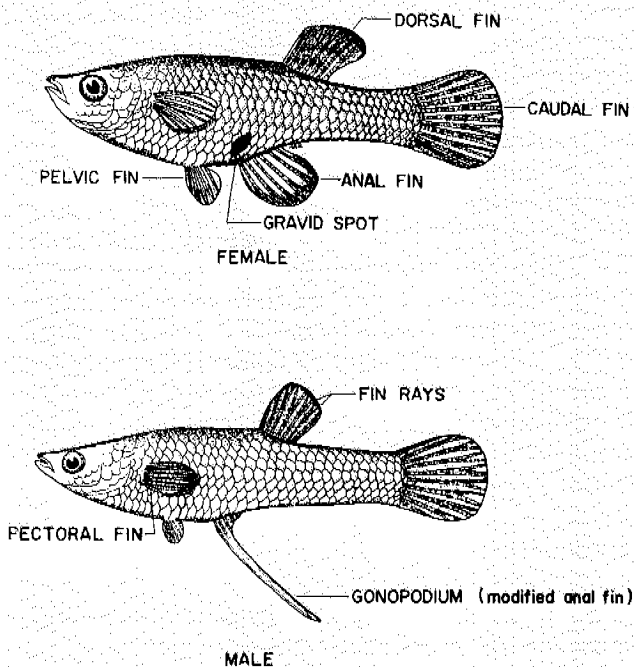

Figure 1. Gambusia affinis.

The average gestation period in **Gambusia** is 23 to 24 days (Krumholz, 1948), but varies with temperature and possibly with other factors. Parturition, or the time required for a single brood to be born, will take about 30 to 60 minutes (Krumholz, 1948). On the average, three to four broods will be produced and, depending on the size of the female, the number of fry per brood may average from 40 (Hildebrand, 1921) to 104 (Hoy and Reed, 1970), although broods of 300 or more may occasionally be encountered. Overlapping of broods does not occur and only a single brood of developing embryos are present in the ovary at any one time. Therefore, the female will have broods throughout the summer at intervals of 3 to 6 weeks, the length of the breeding season varying with the latitude and climate (Hildebrand, 1921). According to Hildebrand (1921), the breeding season in the southeastern U. S. begins in May and ends in September or October, while in Florida (Key West), it occurs all year round, although gravid females are less numerous during the winter months. Hubbs (1971) reports that female **Gambusia** are reproductive from March through September in Texas. In Illinois, Krumholz (1948) found that females present at the onset of summer were virgins born the preceding fall, which became gravid for the first time at an age of 8 to 10 months. However, female offspring of the first, and possibly second, generations became gravid at an age of 4 to 6 weeks. Hubbs (1971) made similar observations in Texas.

According to Krumholz (1948), mosquito fish may live for 4 to 5 years in aquaria, but they seldom survive more than 2 years in the wild. As a general rule, both males and females were found to die the same summer in which they reached maturity. However, the maximum life span was found in females which did not become mature until their second summer. These fish liberated their series of broods and died at an age of 15 months.

EFFECTIVENESS

The effectiveness of **Gambusia** as a mosquito predator has been well documented over a period of 70 years. Characteristics which have contributed to this effectiveness include the following: (1) flattened heads and protrusible mouths which enable the fish to readily prey on surface feeding larvae and pupae; (2) small size which enables them to inhabit shallow waters and penetrate dense vegetation growth where larvae and pupae hide; (3)

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ovoviviparous reproduction which does not require specialized substrates for oviposition as in many egg-layers; (4) high fecundity, short life cycle and voracious appetite for aquatic insects; (5) broad tolerances for temperature changes, salinity, organic pollution, overcrowding and poor food supply; (6) hardiness, adaptability and subsequent ease of handling during transporting; and (7) relative lack of susceptibility to disease.

Gambusia is a hardy species and yet their effectiveness in any given situation will depend on: (1) the amount and type of vegetation and debris present; (2) the density of the fish population; and (3) the existence of natural enemies such as predaceous fish. In situations where dense vegetation or debris provide adequate protection for larvae and pupae, good control may not be obtained. Exceptions to this will occur when an overabundance of fish causes an active search for prey, even in heavily vegetated areas (Hildebrand, 1921). According to Hildebrand (1925), the degree of control obtained will be dependent upon the density of the fish population. When population numbers are small, a comparatively larger number of mosquito larvae will escape than when mosquito fish are in abundance, and the competition for food correspondingly greater. This is particularly true when adequate hiding places exist for larvae and pupae. The existence of natural enemies which prey on Gambusia will tend to keep the population at a level which precludes good control. Gambusia is more effective when introduced into areas where its predators are naturally absent or have been eliminated prior to stocking. These predators may include birds, frogs and larger aquatic insects. In most cases, however, they will be larger carnivorous fish such as pike, bass and sunfish.

Because the effectiveness of **Gambusia** is obviously dependent on the three factors discussed above, it may be necessary to disturb or change one or all of these to obtain adequate control. This is very evident when **Gambusia** and mosquito larvae are found living together in the same areas. As discussed by Barney and Anson (1921), it is necessary to modify the environment of such an aquatic situation in order to magnify the effectiveness of **Gambusia** as a larval predator. This can be accomplished by removing the vegetation, increasing the number of fish by stocking, providing places of refuge, or eliminating natural fish predators.

In Michigan, Krumholz (1948) found in experiments over two summers that Gambusia

were about 81% and 95% effective in controlling anopheline breeding in ponds. In Georgia ponds and swamps, Hildebrand (1925) found mosquito fish to be responsible for a 50% and 80% average reduction in anopheline and culicine populations, respectively. The most effective control of both species was in artificial ponds while control was much less successful in several heavily vegetated swamps. Similar observations have been made by other authors, and it should be realized that absolute or 100% control cannot be expected in every situation. The fish may also be more effective under some circumstances than others.

The mosquito-like chironomid midge, while non-biting, is often an intolerable pest in certain areas. Like mosquitoes, the immature stages are aquatic and it might be expected that Gambusia would also be effective in their control. Bay and Anderson (1966) concluded from studies in California, however, that mosquito fish had little or no value in the control of chironomids.

HUSBANDRY

Mosquito fish "husbandry" has become an accepted term for the manipulation of fish for mosquito control (Fowler, 1972). In this guide, husbandry will include methods for collecting, transporting and stocking mosquito fish along with information on integrated control techniques.

Collecting

In order to establish a mosquito fish program for biological control, a system for mass collection is essential. There are basically two major styles of collecting: active and passive. In active collecting, the fish are actually chased or surrounded in a physical manner, while in passive collecting, a trap is used in which the fish capture themselves. The type of equipment used to accomplish collecting may include aquatic or dip nets, minnow seine nets and a number of different fish traps. In order to select the best method for each individual situation or locality, each of the methods and their advantages and disadvantages are discussed below. A review of the Biology section will indicate those areas to investigate for sources of mosquito fish.

The aquatic net, or dip net, (Figure 2) consists of a small mesh bag on a long wooden handle. It is an inexpensive piece of equipment which is used in active collecting by one man. While an aquatic net might be useful in a small scale operation, its use is

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Figure 2. Aquatic net or dip net

Figure 3. Fifty foot minnow seine or drag net

time consuming, inefficient and inadequate when large numbers of fish are required. In addition, the fish may be seriously stressed as they are scooped from the water.

The minnow seine, or drag net, is suitable for use in most locations as another form of active collecting. These vary in size from small scoops to large drag nets of 6 x 50 feet (Figure 3). The ideal mesh size is 3/16 inch and the addition of 1 1/2 inch x 6 foot dowelling, attached at each end of the seine, will facilitate handling (Fowler, 1972). In shallow water where large populations of Gambusia are present, this method has a definite advantage as large numbers can be easily surrounded and captured. However, there are certain disadvantages: seining may require the assistance of several individuals; it is usually time consuming; and in deep water, such as a reservoir, a boat is required to operate the nets. Stress is also an important factor. When the seines are dragged over muddy bottoms, the fish frequently become fouled with mud which clings to the fins and scales. If not completely washed off, death will usually follow. If the net is dragged too long,

particularly in shallow water, the fish may be injured, leading to death or a fungus infection. Finally, seining is indiscriminate and will capture all species of fish. Sorting will be required before stocking can be accomplished, and this additional handling results in longer exposure to air and subsequent high mortality.

Active methods of collecting have inherent disadvantages and the use of fish traps for passive collection is usually recommended. The wire minnow trap is a popular bait fish trap constructed of small mesh hardware cloth (four or eight mesh) in the shape of a barrel or cylinder, with funnels at either end (Figure 4). The trap is baited inside with dry bread, crackers, or dog food and then positioned in shallow water with both funnel ends submerged. A cord or chain is attached to the trap to anchor it to the bank. Where small populations exist, best results will probably be obtained when the trap is set during the peaks in feeding activity. In areas where populations are heavy, large numbers may be captured throughout the day and within a few minutes after the trap has been positioned.

Where large numbers of Gambusia are required in a program, the "box-style" traps are recommended. The box trap is basically an oversized minnow trap. Caton and Sjogren (1969) described a box trap consisting of a wood frame box covered with 1/8 inch hardware cloth and a hinged top for fish removal. The trap utilized one

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Figure 4. Wire minnow trap

Figure 5. Portable box trap, assembled and disassembled

Figure 6. Positioning of portable box trap

funnel entrance and a lateral weir arrangement to channel the fish into the interior. Stains (1970) developed a similar box trap for use in sewage lagoons. Constructed of redwood two by fours, it was rectangular in shape with dimensions of 3' x 3' x 10'. Two hardware cloth funnels were utilized along with lateral weirs to channel the fish into the trap from either direction.

The permanent box trap is well suited for large activities requiring an extensive mosquito program and having access to a permanent source for collection. However, to fulfill the need for a less permanent and more portable collection system, a modified box trap (Figure 5) was designed which was lightweight, collapsible and easily assembled in the field by one man (Ehrhardt and Sholdt, 1972). This portable trap is described in detail in Appendix A in anticipation that it will be of use to activities not requiring permanent traps and having limited storage facilities.

Because Gambusia is a shoaling species, best results are achieved when the trap is set parallel to, and about 2 to 4 feet from, the shoreline (Figure 6). Obviously, the water level must at least be above the funnel entrances. Conversely, it should not be too far above the entrance holes, as Gambusia normally swim near the surface and will not enter the holes if the trap is too deeply

submerged. The best time for collection is in the early morning and late evening periods when their feeding activity is peaked. In heavy populations, the numbers captured with this box style trap is phenomenal. Stains (1970) roughly estimated that as many as 100,000 fish were collected in a single day using his trap.

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Transporting

To collect the necessary quantity of fish required for effective control, one of several trapping methods can be selected. Similarly, after capture, the fish may be transported in a number of opened or sealed containers such as garbage cans and plastic bags, repectively. Sweetman (1936) recommended using 10 gall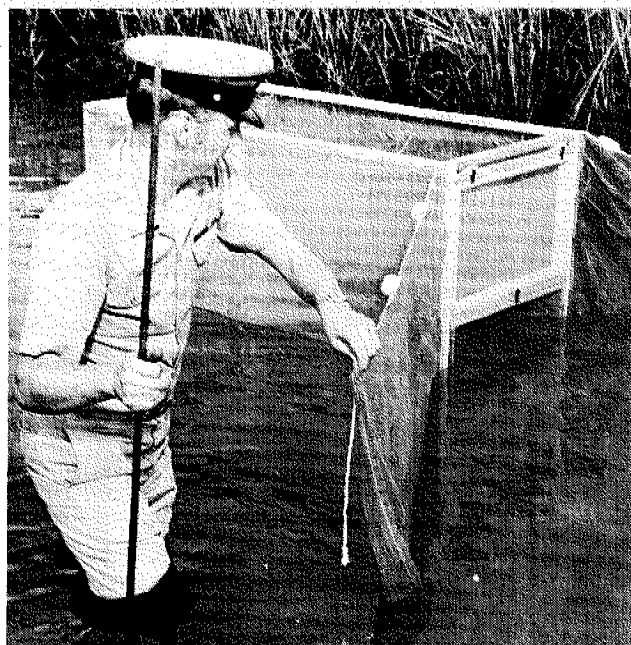on lard tins for transporting 30 to 40 larger-sized fish. In Iran, Tabibzadeh, et al., (1970) transported about 300 fish in 30 to 40 liter polyethylene bags half-filled with water and then pumped full with oxygen and sealed. Nakagawa and Ikeda (1969) successfully transported 2,000 fish in 30 gallon polyethylene storage containers. Aeration was provided using airstones and compressed oxygen. Russell (1970) reported that 10,000 to 15,000 fish could be transported for short periods in 15 gallon garbage cans with lids, provided the water was well aerated. Ice was used to cool the water during extended holding periods in hot weather. When small numbers of up to 200 fish are to be transported for short distances, a commercial minnow bucket or 3 gallon plastic pail is usually adequate. Due to the exceptional jumping ability of Gambusia a cover or screen may be required on open containers to reduce the loss of fish while in transit.

Regardless of the type of container used, oxygen in some form should be supplied to the fish. As pointed out by Lewis (1970), when Gambusia become overcrowded, mutual interference occurs at the surface and each fish periodically loses contact with this oxygen source. Under these circumstances, the fish become less efficient in using surface oxygen. Therefore, high mortality results very quickly as the critical level of overcrowding is reached. This is particularly true during hot days when the fishes' requirement for oxygen is higher, but the amount of oxygen available in the water for respiration has decreased due to the increased water temperature. In open containers, adequate aeration can be achieved using diffusers or airstones made of porous stone. The source of air can be any type of air compressor, electric fuel pump or compressed air cylinder. Tygon tubing is used to connect the diffusers with the source of air. When a stream of fine bubbles is released through the airstone, direct oxygenation occurs, and the water is circulated bringing the poorly oxygenated water in the deeper layers to the surface where oxygen is available at the air-water interface. In deep containers, such circulation is particularly critical. Airstones found in tropical fish stores are sufficient for small containers while the type used in fish hatcheries are necessary for very large containers. The air can be metered using a surgical clamp or an aquarium air valve. Optimum aeration is a heavy stream of fine bubbles. Over-bubbling should be avoided because it creates heavy turbulence and little oxygen is dissolved.

A compact system designed for aeration of fish bait has been used successfully for **Gambusia** transportation. The system, as shown in Figure 7, consists of a small air cylinder, metering valve, air stone and tubing. The cylinder can be charged with air from a service station tire pump and when filled to capacity will deliver air to a container for 5 to 7 hours.
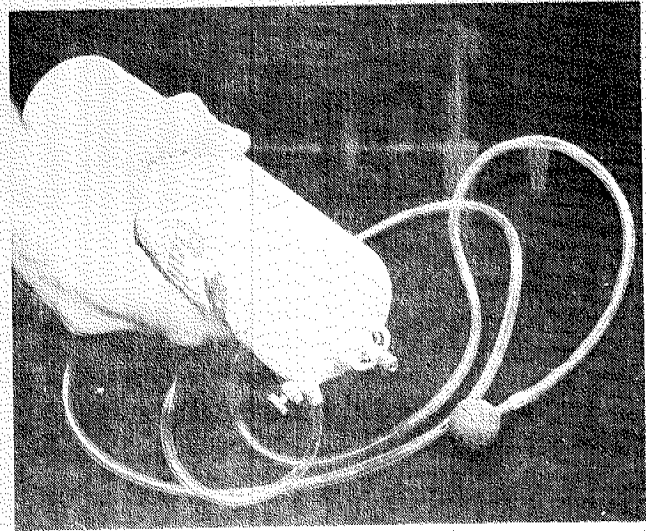
Figure 7. Compact aeration system used for keeping bait fish

The use of plastic bags as sealed containers for the transportation of fish has been known to tropical fish importers for several years. In addition to being inexpensive and suitable for transporting fish long distances, plastic bags have advantages over metal containers in that they: (1) do not rust; (2) prevent injury to fish bumping into container walls; (3) protect against temperature changes; (4) are lightweight but durable; (5) are portable; and (6) require little storage room. Also, the bags readily facilitate water temperature equalization at the stocking site by floating them in the water before releasing the fish (Miller, 1956).

The fish are placed in 5 to 20 gallon, heavy duty plastic bags contained in styrofoam boxes (Figure 8), which provide good insulation against

temperature fluctuations. Each bag is filled about 1/4 to 1/3 full of water and a maximum of about 200 fish per gallon are added. After deflating the bag, pure oxygen is added from a compressed
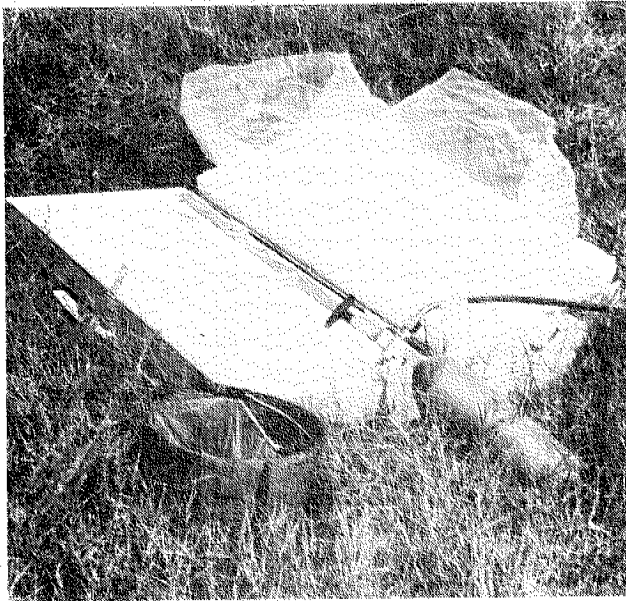

Figure 8. Closed container equipment for transporting mosquito fish

oxygen cylinder (Figure 9), and the top of the bag twisted, bent over and fastened with a rubber band. When the lid of the box is in place, the fish are ready for transporting to the stocking point. For added protection, it may be desirable to use a cardboard box or plywood case to hold the styrofoam boxes during transit.

The plastic bags and styrofoam boxes can often be obtained from local tropical fish dealers at minimum or no cost. A small compressed oxygen cylinder (FSN 6505-132-5181) is usually available from a dispensary. It is important that pure oxygen be used in the plastic bags instead of atmospheric air. Nemoto (1957) found in studies with two different fish species that survival time was increased by 20 to 70 hours when air was replaced with pure oxygen in sealed containers. The maximum holding time using the plastic bag technique will usually be 2 to 3 days; however, the bags must remain fully inflated. Fowler (1972) reported that Gambusia were air shipped from California to Montana using the above technique. The time in transit was about three days with negligible mortality.

Regardless of the method of transportation, the fish should not be artificially fed on the day prior to, and during, actual transportation. Temporary

Figure 9. Adding oxygen to plastic bags

starvation lowers metabolic discharges (Nemoto, 1957) and reduces the amount of regurgitated food that can contribute to pollution. The fish also appear to become more resistant to temperature fluctuations and handling injuries (Norris, et al., 1960). Another critical factor to consider during transportation is water temperature on hot days. In open containers, the addition of block ice may be useful. However, the amount of ice used should be controlled so that the temperature remains within 4 to 5 degrees of the water from which the fish were taken. Sudden and drastic changes in temperature cause undue stress on the fish producing mortality or predisposing them to disease.

Besides temperature, other factors which limit the maximum numbers of fish that can be transported include: oxygen consumption, carbon dioxide production, excretion of nitrogenous wastes and excess activity (Norris, et al., 1960). To control these factors, chemical agents capable of inducing anesthesia or deep sedation in fishes have been studied and used by Fish and Game

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Commissions, tropical fish importers, and commercial bait haulers. These agents, termed general anesthetics, all act to varying degrees as central nervous system depressants (Bell, 1964). According to McFarland (1960), fish anesthetics decrease the fishes' reactions to external stimuli during transportation. Subsequently, hyperactivity decreases causing a reduction in oxygen consumption, and a reduction in the accumulation of carbon dioxide and metabolic waste products. As a result, significantly more fish can be transported than without the use of anesthetics.

Some studies have been made on the use of anesthetics for transporting Gambusia in California (Sjogren, 1971), however, published data is scarce. The limiting factors discussed above are probably less critical for Gambusia, an extremely hardy fish, and it is unlikely that most programs will require the use of fish anesthetics if proper aeration requirements are met. It should be noted, however, that the potential for fish anesthetics for transporting large numbers of Gambusia for extended periods of time is promising. Those wishing to further investigate the subject should review the paper by Bell (1964) which lists the properties and characteristics of several fish anesthetics in a useful chart form, in addition to the references cited above. Certain general anesthetics are extremely hazardous compounds, and their use should not be attempted before consulting with a Fish and Game Commission or other cognizant source.

Stocking

In selecting a suitable stocking site, several factors should be considered. To begin with, the sites where Gambusia often fail include those areas which are too cold, too plant infested, too extensive or too temporary for them to reach control levels or where protection from natural enemies is not adequate (Bay, 1967a). Next, in areas where extremes in summer and winter temperatures occur, ponds with a minimum depth of 5 feet will be required for the fish to survive (Krumholz, 1948; Tabibzadeh, et al., 1970). Thirdly, Krumholz (1948) states that Gambusia are capable of maintaining a population in salt water and, in fact, they have been shown in the laboratory to withstand salinity over twice that of sea water (Ahuja, 1964). But, Harrington and Harrington (1961) consider them to be overvalued in their usefulness for the control of salt marsh mosquitoes, and there is little information available

to the contrary. Their use in salt marsh areas may, therefore, be limited. Finally, in North Carolina, ponds have been observed to support large populations of both game fish and Gambusia with no apparent adverse effects. However, there have been reports of mosquito fish threatening the existence of game fish by feeding on their young fry (Myers, 1965). It is recommended, therefore, that those areas designated for sport fishing not be stocked if a possible decline in game fish will result in adverse command reaction.

Areas in which mosquito fish generally provide adequate control include: irrigation systems, drainage ditches, sloughs, ponds, cisterns, shallow wells, watering troughs, seepage areas, borrow pits, sewage oxidation ponds or lagoons, water hazards on golf courses, undrained swimming pools, and other areas of standing water which allow for mosquito breeding. Gambusia are effective in ornamental fish pools, but are best used by themselves due to their aggressive behavior against other fish species such as goldfish (Myers, 1965). Personnel with state Fish and Game Commissions or the U. S. Fish and Wildlife Service are probably best qualified to assess the ecological situation and specify areas which can safely be stocked with Gambusia It is highly recommended, therefore, that these agencies be contacted before transplanting Gambusia to any new site within the activity or the local area. Such precautions will preclude any unpleasant repercussions from arising later.

While not normally required, artificial feeding may be desirable to achieve maximum potential in some areas. In such instances, the food of choice is "trout chow" such as fish hatcheries use. The amount of trout chow necessary for good growth will be dependent upon water temperature, the availability of natural food, and the size and total numbers of fish to be accomodated. Prior to stocking, the elimination of predaceous fish and other natural enemies of Gambusia may on rare occasions be necessary. Obviously, the killing of fish for any reason can have serious repercussions if accomplished improperly or without good justification. Therefore, coordination of such a project must again be made through the command, the activities' fish and wildlife representative, and appropriate medical department and public works personnel. Recommendations and approval for an appropriate chemical should be obtained from the cognizant Naval Facilities Engineering Command Applied Biologist.

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After a site has been selected, approved, and properly prepared, the number of fish to be stocked will be governed by the surface area of water and the amount of protection available to the mosquito population. Overstocking is usually not a critical factor. Therefore, when fish are readily available, the higher the number stocked, the quicker the degree of control. Each situation will vary and as a guideline, the rates used by other workers are reviewed. Hoy, et al., (1971) found that good mosquito control was achieved in California rice fields with stocking rates of 300 mature females per acre when applied early in the season. In Louisiana, Craven and Steelman (1968) achieved 96% control of mosquito larvae populations in flooded rice fields stocked at rates of one half fish per square foot of water surface. Tabibzadeh, et al., (1970) found that fifteen females and one male per square meter of water surface gave good control in extensive breeding areas of Iran, while for small water collections with no vegetation, two females and one male per square meter were adequate. Before releasing the fish, the temperature of the water in the container and that of the stocking site should be equalized, particularly if a difference of more than 4 to 5 degrees F. exists. This can be accomplished by floating the container in the water or gradually adding water from the stocking site to that of the container.

Nakagawa and Ikeda (1969) reported on two methods utilizing helicopters for stocking fish in inaccessible areas. In one instance, storage tubs were lashed to the skids of a helicopter and the fish scooped out by hand and dispersed as the aircraft hovered from site to site. In another case, the fish were placed in a spray tank from which the spray system had been removed. The fish were dispelled from the aircraft by opening the main valve on the tank. In California, 1 gallon plastic bags were filled with water, approximately 200 fish, and a rock, and then released over stocking areas from a helicopter at heights of 20 to 30 feet. The weighted bags sank to the bottom where they opened and released the fish (Wolfe, 1970).

The amount of time required for a population to reach a level sufficient to provide effective control will depend on the number stocked initially. When minimum numbers are planted (i. e., 100 to 300 females per acre), a period of 30 to 60 days may be required before the population reaches an effective level. It is, therefore, advisable to stock as early in the season as possible such as in April or May. After stocking, the populations may reproduce at phenomenal rates. Fowler (1964) reported that within 30 days, 4,000 to 8,000 fish were obtained from rice fields initially stocked with 200 mature females. Russell (1970) estimated that a total of 1.5 million fish were removed over a 3 year period from one pond initially stocked with 2,000 Gambusia.

Integrated Control

The management of pest populations through the use of integrated control techniques has received increased attention in the past few years. The term "integrated control" is usually defined as the use of chemical, biological, and physical control measures either in sequence or simultaneously against a pest population. In a mosquito fish program, the central theme consists of using chemicals, namely larvicides and herbicides, to augment the biological control efforts of the fish; thereby assisting in the achievement of adequate mosquito control. The larvicides are applied early to control new mosquito broods, and then they may be discontinued when the fish multiply and become fully effective (Bay, 1967b). Herbicides, on the other hand, serve to control dense vegetation where mosquitoes see refuge from the predatory Gambusia.

Obviously, the choice of insecticide is critical since a selective material is required which will kill the mosquitoes but spare the fish population. At field dosages, Lewallen (1959) found Thimet, Guthion, malathion and parathion to produce high mortalities in mosquito fish. Similarly, chlordane, endrin, heptachlor, Thiodan, toxaphene, aldrin, dieldrin and isodrin were found to be hazardous to Gambusia (Mulla, 1963). Compounds found to be relatively safe against mosquito fish at mosquito larviciding rates include: Abate (Hamon, 1970); fenthion (Mulla and Isaak, 1961; Patterson and von Windeguth, 1964; Nakagawa and Ikeda, 1969); naled (Lewallen, 1959; Mulla, et al., 1963); Dursban (Ferguson, et al., 1966); methyl parathion (Mulla, et al., 1963); and FLIT MLO (Welsh, 1972). Diesel fuel will probably not kill fish at low rates but it may have some repellent action. Hildebrand (1921) suggests that diesel fuel is disagreeable to the fish and may destroy some of their food. Also of interest is the reported resistance or tolerance of mosquito fish to a number of the organochlorine insecticides (Boyd and Ferguson, 1964a, 1964b; Hamon, 1970).

Hildebrand (1921, 1925) felt aquatic plants with slightly submerged leaves or with a dense network

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of roots near the water surface offered the best protection to immature mosquitoes from fish. Algal mats were also considered to provide good refuge when water found in portions of the mat was sufficient to harbor mosquitoes. **Anopheles** larvae have been found in the thin film of water that covers partially submerged leaves. In such instances, they cannot be detected or reached by larvivorous fish (Hildebrand, 1919; Miller, et al., 1970).

Whenever possible, it is recommended that the vegetation be removed mechanically. If, however, a herbicide is required then a number of factors (Sims, 1972) must be considered: (1) if the plants are growing in the shallow edges or margins, or on the bank above the water line; (2) if the water is free-flowing as in a canal or static as in a pond; (3) if the vegetation is a grass or a broadleaf and if it is submerged or immersed: (4) if the herbicide will be compatible with the aquatic animal life; and (5) if the treated water will be used for drinking by livestock, wildlife or humans. General guidelines for aquatic weed control are contained in Agricultural Handbook No. 332 (U.S. Department of Agriculture, 1969) and NAVFAC Manual MO-314 (U.S. Department of Defense, 1970).

Final approval and assistance should be obtained from the cognizant Applied Biologist, Naval Facilities Engineering Command.

If compatible, the appropriate herbicide and insecticide may be used in combination to selectively control vegetation and mosquito larvae at the same time. It should be emphasized that the use of larvicides and herbicides be initiated only when and where necessary. Chemicals will not be required if the fish are keeping the mosquito population under control or adequate vegetation is unavailable for the mosquitoes to find refuge.

Other methods of improving or facilitating integrated control would be to construct drains leading to ponds stocked with fish and to thin or remove vegetation with herbivorous or omnivorous fish, such as carp. In Hawaii, **Gambusia** were used in combination with an algae-feeding fish, **Tilapia mossambica.** As the **Tilapia** multiplied, their feeding cleared surface algae enabling **Gambusia** to successfully attack mosquito larvae (Nakagawa and Hirst, 1959). This type of biotic activity has been termed "bio-synergistic" by Gerberich and Laird (1968).

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APPENDIX A
CONSTRUCTION OF A PORTABLE BOX TRAP

The portable box trap was patterned after the one described by Stains (1970). It was constructed of 3/4" X 1 5/8" Cypress slats covered with eight mesh or four mesh hardware cloth. The eight mesh (1/8 inch) hardware cloth was generally used for collecting large numbers of males and females. In cases where only the larger, mature females were desired, a trap covered with four mesh (1/4 inch) hardware cloth was used, as the smaller males and immature females easily passed through the screen. Approximately four man hours were required to complete the trap at a total cost of about twenty-five dollars.

The trap was constructed in a series of steps (Figure 10) to insure a proper fit of all pieces. In step A, the two trap sides were completed with outside measurements of 35 1/4" X 55 1/2". All corners were joined using 2 1/2" finishing nails and secured with polyvinyl resin white glue. The hardware cloth was trimmed to the proper dimensions and attached with a hand-operated stapler and 1/2" staples.

The two trap ends, step B, measured 25 1/2" X 35 1/4". Double wood slats were utilized at the top and bottom of the trap ends to allow space for the turn buttons to function. The center slat was carefully measured in order that the funnel and flat inserts could be freely interchanged. Finally, small strips of hardware cloth were stapled in place over the double slats.

The bottom of the trap was completed in step C, after minor adjustments were made to insure a uniform fit of the side and end pieces. After the hardware cloth was secure, the trap sides and ends were placed in position upon the bottom piece. By taping the parts together with masking tape, the hook and eye latches could be accurately secured. Fourteen latches were used; two in each corner and six along the bottom. To hold the funnel and flat inserts in place, a total of ten turn buttons were fastened to both sides of each trap end as shown in Figure 10, B.

In step D, four rectangular frames were constructed with outside measurements of 14 7/8" X 23 7/8". Two of the frames were fitted with hardware cloth to make the flat inserts. For each funnel insert, two triangles of hardware cloth measuring 13 7/8" X 15" and two measuring 12" X 23" were cut (Figure 10, E and F). The base lines of the four triangles, which corresponded to the inside measurement of the rectangular frames, were stapled in place. Wax covered string was used to draw the sides together into a pyramid. The string was tied at the base of each corner and then woven upward through the hardware cloth using a chain stitch.

Diverging lateral weirs made of minnow seine nets were used to direct the fish into the trap. Two 4' X 10' seine nets were cut in half for each end of the trap and fastened in place with 1/2" staples. Unlike hardware cloth weirs, the seine nets readily conformed to the contoured beds of collecting areas, were easily installed, and reduced the bulk and weight of the trap.

The trap worked equally well in shallow and deep water. Approximately fifteen minutes were required for assembly. In shallow water, the funnel inserts were placed in the lower trap openings. After collection was completed, they were carefully exchanged with the flat inserts. By quickly installing the flat insert behind the funnel as the latter was pulled up through the interior, the fish could be removed with no interference from the protruding cones. In deep water, the funnel inserts were used in the upper openings while the flat inserts closed off the lower. After the trap was pulled close to shore, removal of the captured fish was easily accomplished using a large tropical fish net.

A. Side.

B. End. X = turn buttons.
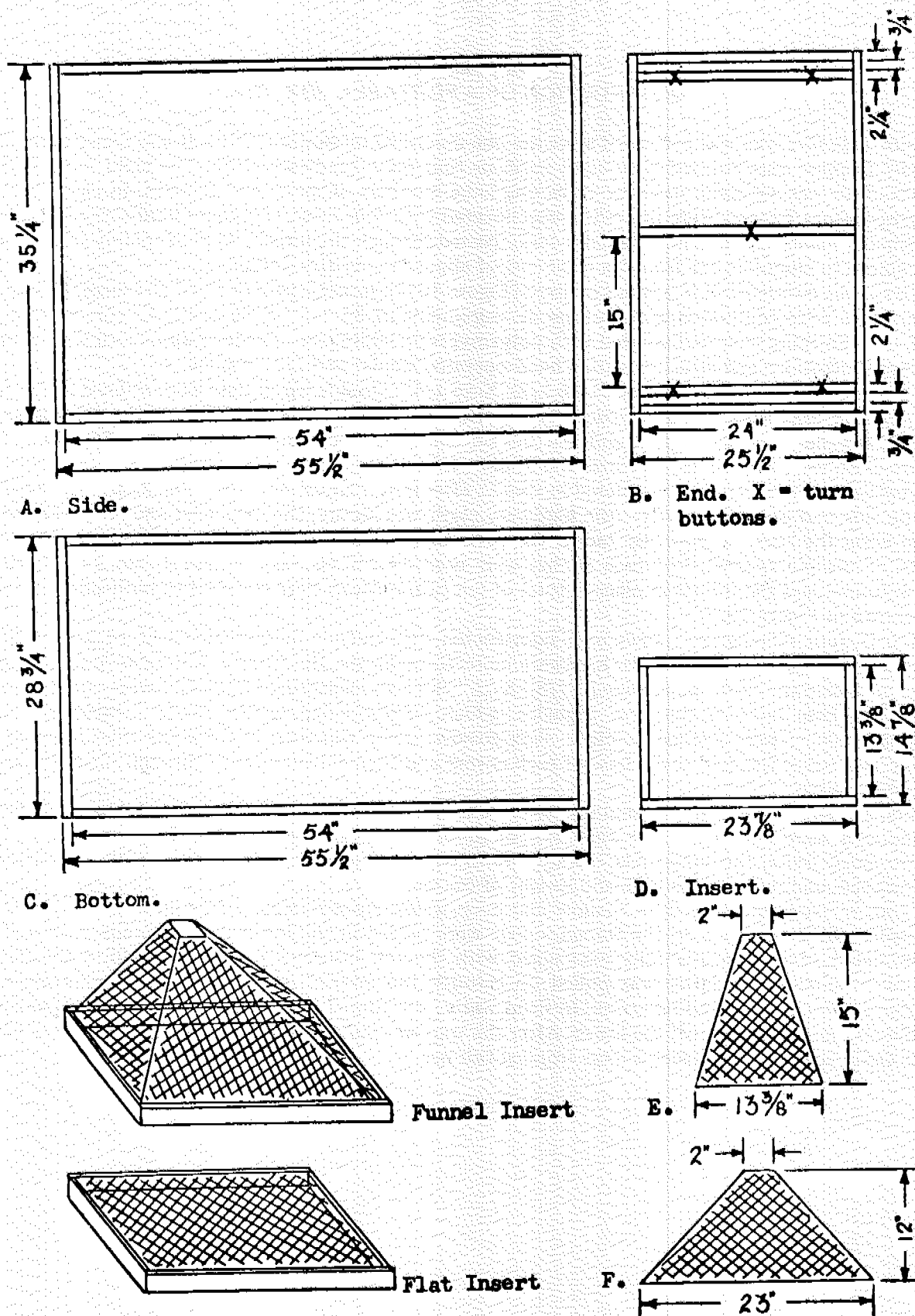
C. Bottom.

D. Insert.

Funnel Insert

Flat Insert

E.

F.

Figure 10. Specifications for construction of a portable box trap

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MOSQUITO FISH PROGRAM

NEPMU-2